510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
k040534

B. Analyte:
alpha-Amylase

C. Type of Test:
Quantitative

D. Applicant:
Clinical Data, Inc

E. Proprietary and Established Names:
Vitalab Alpha-Amylase Reagent Kit

F. Regulatory Information:

1. Regulation section:
   21 CFR 862.1070
2. Classification:
   Class II
3. Product Code:
   JFJ
4. Panel:
   Chemistry (75)

G. Intended Use:

1. Intended use(s):
   The Vitalab Alpha-Amylase Reagent Kit is a device for the quantitative measurement of alpha-amylase activity in serum and plasma.
2. Indication(s) for use:
   Amylase measurements may be used for the diagnosis and treatment of pancreatitis (inflammation of the pancreas).
3. Special condition for use statement(s):
   Prescription use
4. Special instrument Requirements:
   The Vitalab Amylase Reagent is intended to be used with the Vitalab Selectra E Chemistry Analyzer.

H. Device Description:
The Vitalab Amylase Reagent and the Vitalab Selectra E Analyzer are used as a system for the quantitative analysis of alpha-amylase in serum and plasma. The Vitalab Amylase Reagent is

supplied as a single part liquid-stable reagent. The reagent components are: 2.25 mmol/L 2-chloro-4-nitrophenyl-α- D -maltotrioside, 300 mmol/L sodium chloride, 6.00 mmol/L calcium chloride, 900 mmol/L potassium thiocyanate, 50 mmol/L MES buffer. Vitalab Serum Control Kit is required but not provided with the reagent. There are no calibrators with this assay.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Roche alpha-Amylase EPS Ver.2 Reagent, product 03183742
2. Predicate K number(s):
   k972250 (reagent was cleared with Cobas Integra analyzer)
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	Quantitative determination of alpha-amylase in serum and plasma	Quantitative determination of alpha-amylase in serum, plasma and urine
Measurement method	Kinetic, measured at 405 nm	Kinetic, measured at 409 nm
Analytical Range	5 to 1,600 U/L (default range) 1,200 to 3,200 U/L (rerun range)	0 to 2,000 U/L 0 to 10,000 U/L (with post dilution)
Differences		
Item	**Device**	**Predicate**
Chemical Reaction	Cleavage of 2-chloro-4-nitrophenyl-α-D-maltotrioside to produce 2-chloro-4-nitrophenol	Cleavage of 5-ethylidene-G7PNP to produce G_nPNP, which is detected by an indicator reaction to produce 4-nitrophenol
Reagent components	2-chloro-4-nitrophenyl-α-D-maltotrioside, sodium chloride, calcium chloride, potassium thiocyanate, MES buffer	5-ethylidene-G7PNP sodium chloride, calcium chloride magnesium chloride α-glucosidase HEPES buffer
Calibration	Assay requires no external calibration material	Single point serum calibrator

J. Standard/Guidance Document Referenced (if applicable):

The sponsor cites NCCLS publications EP3-T, *Tentative Guidelines for Manufacturers for Establishing Performance Claims for Clinical Chemistry Methods, Replication Experiment*, and NCCLS Document EP7-P, Volume 6 No.13. (Osmotic Shock Procedure)

K. Test Principle:

The Vitalab Amylase Reagent determines alpha-amylase through the cleavage of 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNP-α-G_3) to produce 2-chloro-4-nitrophenol (CNP), a yellow dye. Results are calculated from rate of increase in absorbance of CNP at 405 nm.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision using both the default and rerun sample volumes is demonstrated by the replicate assay of commercially available control serum. These controls are spiked with higher concentrations of alpha-amylase (from porcine pancreas) for the rerun precision study. Each sample is assayed in triplicate in twenty runs over at least 10 days using the Vitalab Amylase Reagent on a Selectra E Analyzer. Precision statistics, calculated analogous to the method described in NCCLS Guideline EP3-T, are shown below.

Precision of Amylase Recoveries in U/L

Instrument Range			Within Run		Total	
Sample	n	mean	1SD	%CV	1SD	%CV
Default						
Serum 1	60	98	1.0	1.0%	1.5	1.5%
Serum 2	60	565	3.6	0.6%	5.0	0.9%
Serum 3	60	1,034	7.0	0.7%	9.7	0.9%
Rerun						
Serum 1	60	1,961	17	0.9%	25	1.3%
Serum 2	60	2,517	26	1.0%	41	1.6%
Serum 3	60	3,002	40	1.3%	50	1.7%

b. Linearity/assay reportable range:

Linearity is shown for the default and rerun ranges in separate studies. The claimed linearity/reportable range for the assay is 5 – 1600 U/L (default range) and 5 – 3200 U/L (rerun range). The extended range on rerun is accomplished by reducing the sample size by 50%. To demonstrate linearity for the **default** range, seven amylase linearity standards are prepared by quantitatively diluting a stabilized stock solution of alpha-amylase (from porcine pancreas) with a buffered solution. These standards are assayed on a single Selectra Analyzer in ascending order over four analytical runs. Standard recoveries are compared to their dilution factors by least squares linear regression which is forced through the origin. A residual statistic is calculated for each standard as the difference between the mean recovery and its predicted value from the regression statistics. The maximum residual is less than 5 U/L of amylase indicating linearity to at least 1645 U/L, which is the level of the highest standard. To demonstrate linearity for the **rerun** range, twelve amylase linearity standards are prepared as described above. These standards are assayed on a single Selectra Analyzer in ascending order over five analytical runs. Standard recoveries are compared to their dilution factors by least squares linear regression which is forced through the origin, and a residual statistic is calculated for each standard as described above. The maximum residual is less than 12 U/L of amylase indicating linearity to at least 3311 U/L, which is the level of the highest standard.

c. Traceability (controls, calibrators, or method):

The sponsor states that the method is traceable to NIST SRM 938.

d. Detection limit:

Normal saline is assayed thirty times in a single analytical run. The detection limit is calculated as the mean plus two standard deviations of the results. The observed mean and standard deviation are 0.03 and 0.18 U/L respectively. The calculated detection limit of 0.40U/L amylase is rounded up to 1 U/L, which is the round-off error of the assay. The claimed detection limit is 5 U/L.

e. Analytical specificity:

Potential interference from icterus (bilirubin), hemolysis (hemoglobin) and lipemia (triglycerides) is determined in three separate studies. In each study, a serum pool with approximately normal amylase levels is prepared from individual patient specimens and is divided into two aliquots. One aliquot is spiked with the potential interfering substance. The other aliquot is diluted with normal saline, if necessary, to mimic the dilution the spiked pool. These aliquots are then blended to prepare test pools with the interferant concentrations listed below. The red blood cell (RBC) hemolysate, which is used to spike the high pool for the hemolysis test, is prepared from at least five patient specimens according to the Osmotic Shock Procedure described in NCCLS Document EP7-P, Volume 6 No.13.

Interfering Substance	Levels tested
Ditaurobilirubin	8, 16, 24, 32, 40 mg/dL (as bilirubin)
RBC hemolysate	40, 80, 120, 160, 200 mg/dL (as hemoglobin)
Intralipid, 20%	400, 800, 1,200, 1,600 and 2,000 mg/dL (as triglycerides)

Each set of original and spiked pools is assayed in an alternating order 9 and 6 times respectively in a single analytical run. Differences in recoveries between the original and spiked pools are reported with t-statistics. Statistically significant differences greater than 5 U/L are reported in the package insert.
Bilirubin above 8 mg/dL suppresses results by approximately 8 U/L.
Red blood cell hemolysate added to hemoglobin concentrations of 120 and 200 mg/dL decreases recoveries by 7 and 10 U/L respectively.
The addition of Intralipid to 2000 mg/dL triglycerides suppresses recoveries by 5 U/L.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Fifty eight fresh serum and 55 fresh heparinized plasma specimens, selected randomly from individual adult patients, were supplemented with seven abnormal specimens, some of which were purchased frozen, to yield a total of 60 serum and 60 plasma specimens. These specimens were randomly assorted into groups of 15 serum and 15 plasma specimens each. These groups are assayed over four runs using the Vitalab α-Amylase Reagent on the Selectra E and the Roche α-Amylase EPS Ver.2 Reagent on the Cobas Integra 400. Seven specimens were re-assayed in a fifth run because the Cobas results were suppressed due to inadequate sample volume. The serum results, plasma results and the combined results for both specimen types are each compared by Deming regression assuming equal variances between methods. Regression statistics are given below.

Serum Correlation

	Value	95% Confidence Interval
Intercept	-7.7 U/L	-9.5 to -6.0 U/L
Slope	1.097	1.087 to 1.108
$s_{y.x}$	3.7 U/L	
n	60	
range	31 to 1049 U/L	

Plasma Correlation

	Value	95% Confidence Interval
Intercept	-1.7 U/L	-3.7 to 0.4 U/L
Slope	1.034	1.014 to 1.055
$s_{y.x}$	3.6 U/L	
n	60	
range	26 to 394 U/L	

Combined Correlation

	Value	95% Confidence Interval
Intercept	-6.1 U/L	-7.4 to -4.7 U/L
Slope	1.086	1.076 to 1.096
$s_{y.x}$	4.0 U/L	
n	120	
range	26 to 1049 U/L	

Where x = Predicate Results and y = Selectra Results

b. Matrix comparison:
Serum and plasma specimens are individually compared to the predicate method. Deming regression statistics suggest that the serum results are approximately 6% higher than plasma results. Although, this difference is small relative to the clinical requirements of the test, further analysis suggests that it is caused by differences in the distribution of the abnormal serum and plasma samples. This is supported by Passing & Bablok regression, which is less affected by extreme values. These statistics are tabulated below.

Specimen	y-intercept (95% CI)	slope (95% CI)
Serum	2.7 U/L (0.0 to 5.4 U/L)	0.958 (0.917 to 1.000)
Plasma	0.0 U/L (0.0 to 2.1 U/L)	1.000 (0.963 to 1.000)

3. Clinical studies:
   a. Clinical sensitivity:
   Not applicable
   b. Clinical specificity:
   Not applicable
   c. Other clinical supportive data (when a and b are not applicable):
   Not applicable

4. Clinical cut-off:
   Not applicable
5. Expected values/Reference range:
   The published reference range for α-amylase, measured at 37C is 22 – 80 U/L.
   Tietz Textbook of Clinical Chemistry, Third Edition, W.B. Saunders Company, Philadelphia, PA, (1999)

M. Conclusion:

Based upon the information provided for the file, I recommend that the Vitalab Alpha-Amylase Reagent Kit be found substantially equivalent to the predicate device.